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(57) Abstract

To produce plants having modified growth characteristics, target plants are transformed with a DNA construct including a DNA sequence encoding phytoene synthase, an enzyme involved in carotenoid biosynthesis. From the transformants, plants which have modified growth characteristics are selected. Such plants may have a reduced height compared with an untransformed plant or may show other modified growth characteristics, such as an altered pattern of branching or budding.

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PLANTS HAVING MODIFIED GROWTH CHARACTERISTICS

This invention relates to the use of recombinant DNA technology to control the growth of plants.

The use of agrochemicals to alter plant growth characteristics is known. Such chemicals are exogenously applied to the plant and act as plant growth regulators. Many interfere with production of the gibberellin hormone group (plant growth regulators expressed naturally within plants) to obtain the desired effects on plant stature. For example, European Application Publication Number 212822 describes use of an inhibitor of gibberellin synthesis to control tree height. A major group of gibberellin pathway inhibitors are those which function as kaurene oxidase inhibitors, for example 1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-y)pentan-3-ol and stereoisomers thereof including paclobutrazol. The biochemical and biological effects of kaurene oxidase inhibitors have been described by Dalziel et al in British Plant Growth Regul Group, 1984, 11 (biochem aspects synth nat occurring plant growth regul), p43-57 (Chem Abs, 1985, 102(3):249, abstract no 19513r). European Application Publication Number 252617 describes the use of plant growth regulators which inhibit the gibberellin pathway to improve the viability of plantlets on transfer from in vitro micropropagation conditions to in vivo conditions. The plant growth regulator may have a number of beneficial effects: it may reduce the stomatal aperture and influence the stomata's ability to close; it may produce thicker guard cell membranes;

it may produce better formed chloroplasts; it may provide thicker cuticular wax; it may stimulate root formation and favourable root habit; it may have a greening effect; it may provide more numerous and better developed leaf hairs; it may reduce internodal length, providing a more favourable habit in the mature plant; it may ensure that leaf number and size are normal; it may protect from frost damage.

Plants in which gibberellin biosynthesis has been inhibited show a reduction in height, which may be due to reduced elongation of the stem internodes. Other effects on growth may include altered branching and budding patterns. Traditional plant breeding is also used to obtain such dwarf plant varieties adapted for certain purposes.

The ability to produce dwarf plants is particularly useful in certain circumstances. For example, it provides a means to adapt a plant to its environment (for example, so it will not outgrow its limited space and resources);
to improve or control the shape, size and appearance of ornamental plants (including pot plants, shrubs and trees);
to reduce the need for or frequency of trimming or pruning (for example, for trees growing beneath overhead power cables);
to increase stand strength (for example, plants with shorter stems may be less susceptible to wind damage or lodging); and/or
to improve the viability of young plants during micro-propagation.

Plants contain a wide range of biochemicals (including plant growth regulators) which have a direct or indirect effect on phenotype. Gibberellins are isoprenoids and are known to share a common early pathway with other biologically important isoprenoids such as sterols, terpenoid quinones and carotenoids (See Figure 1 in Bramley and Mackenzie, 1988, Current Topics in Cellular Regulation, 29:291-343). Carotenoids include a range of biochemicals such as carotenes, lutein, xanthophylls, and pigments such as lycopene. The biosynthesis of carotenoids in higher plants and micro-organisms has been the subject of numerous investigations. The pathways of carotenoid biosynthesis across a range of organisms share many common features (Bramley and Mackenzie, 1988). The biochemical pathway leading to carotenoid biosynthesis is shown in Figure 1.

The divergence to the dedicated pathway for carotenoid synthesis is after the formation of geranylgeranyl diphosphate (GGDP - previously known as geranylgeranyl pyrophosphate, GGPP). The first unique step in the formation of carotenoids is the head-to-head condensation of 2 molecules of GGDP to form the first C_{40} carotenoid, phytoene. The synthesis of phytoene from GGDP involves two reaction steps with prephytoene diphosphate (PPDP - previously known as prephytoene pyrophosphate, PPPP) as the intermediate, but both reactions are catalysed by a single enzyme in plants and micro-organisms. This enzyme, which catalyses the conversion of GGDP to phytoene, is known as phytoene synthase (Bramley et al, 1992, Plant Journal, 2:343-349).

Phytoene synthase activity has been detected in a wide range of plant species including tomato, pepper, maize, carrot, spinach and tobacco. There are many similarities between the enzymes from different species. The enzymes are located in the plastid stroma and synthesise the same phytoene sterio-isomer (15-cis phytoene). The enzymes all require Mn^{++} as a co-factor although the requirement for ATP is unclear.

DNA sequences encoding phytoene synthase enzymes are known. For example, the TOM5 gene encodes an enzyme involved in the ripening of tomatoes (Ray et al, 1987, Nucleic Acids Research, 15:10587) which has been identified as the enzyme phytoene synthase (international patent application published as WO91/09128; international patent application published as WO92/16635). Phytoene synthase enzymes show sequence similarity. The molecular weight of the purified pepper (Capsicum annuum) phytoene synthase (48kD) (described by Dogbo et al 1988) is very similar to the predicted molecular weight of the mature phytoene synthase protein encoded by TOM5 (47.6kD). Alignment of the predicted phytoene synthase protein sequences from Arabidopsis and pepper cDNA clones with the predicted protein sequence of the tomato phytoene synthase encoded by TOM5 shows 72% and 85% homology respectively. The protein encoded by the tomato TOM5 cDNA also shows significant homology (27% identity; 17% similarity) to the CrtB protein from Rhodobacter capsulatus, a gram-negative purple bacterium (Armstrong et al, 1990, JBC, 265:8329-8338). The CrtB protein catalyses the conversion of GGDP to phytoene (Bartley GE et al, 1992, J Biological Chem, 267:5036-5039).

There is homology between plant phytoene synthase DNA sequences. Tomato contains at least one other locus with homology to TOM5. TOM5 cDNA will cross-hybridise with two clones: the genomic clone known as Clone F (Ray et al, 1992, PMB, 19:401-404) and the cDNA clone known as Psy2 (Bartley and Scolnik, 1993, J Biol Chem, 268:25718-25721). Psy2 has 80% overall nucleotide homology to TOM5 cDNA. The TOM5 cDNA sequence has also been used as a hybridisation probe to identify phytoene synthase clones from other species, including Capsicum annuum which has 83% overall homology (Romer et al, 1993, Biochem Biophys Res Commun, 196:1414-1421) and Arabidopsis thaliana which has 65% overall homology (Scolnik and Bartley, 1994, Plant Physiology, 104:1471-1472).

International application WO91/09128 describes "antisense" and "sense" DNA constructs encoding phytoene synthase and their use to modify the synthesis of carotenoid compounds in plants. These DNA constructs comprise a DNA sequence homologous to some or all of the TOM5 gene, preceded by a transcriptional initiation region operative in plants so that the construct can generate mRNA in plant cells. The DNA construct may encode mRNA which can be translated to give the enzyme produced by the TOM5 gene. International application WO91/09128 describes a method for modifying the production of carotenoids in plants by transforming such plants with the aforementioned DNA constructs, and also describes transformed plants (and their descendants) having a modified carotenoid content. Generally, production of the TOM5 enzyme (phytoene synthase) within the plant is enhanced by

constructs which contain DNA homologous to the substantially complete gene. For example, promotion of lycopene production may be brought about by inserting one or more functional copies of the TOM5 cDNA, or of the full-length TOM5 gene, under control of a functional plant promoter into a tomato plant to give fruit of a deeper red colour. Fray and Grierson (1993, Plant Mol Biol, 22:589-602) discuss the over-expression of plant phytoene synthase genes in tomato and the effect on colour.

In summary, international application WO91/09128 describes the control of TOM5 gene expression in plants (using recombinant DNA technology) to modify colour production and certain other related functions (ß-carotene and Vitamin A production; protection against high light intensity damage; control of sporopollenin synthesis and pollen formation/maturation). We have now observed that over-expression of the TOM5 gene product may affect the growth of plants (a function which is apparently unrelated to the role of the TOM5 gene product).

According to the invention, there is provided a method to produce plants having modified growth characteristics which comprises transformation of target plants with a DNA construct including a DNA sequence expressing phytoene synthase, growth of transformed plants, and selection of plants having modified growth characteristics.

The invention further provides a plant having modified growth characteristics which comprises a plant cell transformed with a DNA

construct including a DNA sequence expressing phytoene synthase, and the progeny and seed of such a plant.

The method according to the invention is particularly useful for producing dwarf plants. A dwarf plant is defined as a plant having reduced height compared with an untransformed plant of the same genotype. This may be due to reduced elongation of the stem internodes. The plant may also show other modified growth characteristics, such as an altered pattern of branching or budding.

Transformation with suitable DNA constructs results in over-expression of the phytoene synthase enzyme in the target plant which has a surprising phenotypic effect on plant stature: the transgenic plants have reduced height and a general dwarf structure. Thus, manipulation of a plant to over-express an enzyme of carotenoid biosynthesis unexpectedly produces a plant with altered growth characteristics similar to those of a plant in which gibberellin levels are reduced.

Over-expression of the phytoene synthase enzyme may be achieved by:
introduction of a phytoene synthase gene into a plant cell where the enzyme is not naturally expressed;
introduction of one or more extra phytoene synthase genes into a plant so that it contains multiple copies of the gene;
enhancing expression of the phytoene synthase gene (for example, the promoter may be selected to give a higher degree of expression than is given by the natural phytoene synthase promoter).

For increased expression of phytoene synthase activity, it is necessary to produce an active protein that catalyses the correct chemical reaction and is directed to the correct location within the cell. Since there is considerable biochemical and sequence similarity between phytoene synthases from different plant species, production of the TOM5-encoded phytoene synthase in transgenic plants and their descendants may be used to modify plant growth characteristics of any plant species. Phytoene synthase-encoding sequences derived from other plant species (for example, those isolated using TOM5 as a probe) may similarly be used.

The method according to the invention provides the ability to produce dwarf plants without the need to apply exogenous agrochemicals. Although it is possible to breed the reduced stature trait into plants using traditional methods, the present invention provides a means of transferring the trait into elite lines without a prolonged breeding programme which might alter other desirable traits at the same time.

International application WO91/09128 demonstrated that the pathway of carotenoid biosynthesis can be effectively regulated by either down-regulating or over-expressing the phytoene synthase enzyme encoded by TOM5. The present invention relates to the use of a DNA construct encoding phytoene synthase to alter plant growth characteristics. Apparently the plant responds to phytoene synthase over-expression by altering the pathway leading to gibberellin production even though said enzyme is not directly involved in the

biosynthesis of said plant hormone. As part of the biochemical pathway leading to carotenoid synthesis, mevalonic acid is converted into geranylgeranyl diphosphate (GGDP). This substrate is used for the synthesis of both coloured carotenoids and gibberellins. Thus the over-expression of phytoene synthase in the carotenoid pathway may alter the levels of GGDP such that the synthesis of gibberellins is reduced.

Observations have shown that transformation with various phytoene synthase constructs has a range of effects on plant growth characteristics. For example, if phytoene synthase expression is inhibited by insertion of antisense or partial sense genes into the plant, it is possible to select transformants having modified growth characteristics consistent with those expected in a plant with elevated levels of the plant hormone gibberellin (such as increased height, precocious germination).

The target plant cell may be part of a whole plant or may be an isolated cell or part of a tissue which may be regenerated into a whole plant. The target plant cell may be selected from any monocotyledonous or dicotyledonous plant species. Suitable plants include fruit-bearing plants (such as tomato, peaches), vegetables (such as potatoes), other crop plants (such as corn, wheat, rice, sorghum, other cereals, oil seed rape, sunflower), ornamental plants (including shrubs and trees), and other tree species (including timber species such as pine, trees used for paper making such as eucalyptus and poplar). Application of the method is not limited to the species listed above.

For example, the method according to the invention may be advantageously applied to potatoes. Inhibiting gibberellic acid (GA) is believed to promote tuber formation (Vreugdenhil and Helder, 1991, Progress in Plant Growth Regulation, Kluwer Academic Press, London, p393-400). Sprouting of mature/stored tubers may also be inhibited in plants with reduced GA levels. (In addition, lycopene production, involving the phytoene synthase enzyme, may occur in the tuber plastids).

Both monocotyledonous and dicotyledonous plant cells may be transformed in various ways known to the art. In many cases such plant cells may be cultured to regenerate whole plants which subsequently reproduce to give successive generations of genetically modified plants. Any suitable method of plant transformation may be used. For example, dicotyledonous plants may be transformed by Agrobacterium Ti plasmid technology, such as described by Bevan (1984, Nucleic Acid Research, 12:8711-8721).

The transformed plants may be reproduced sexually (selfed or crossed) or by cell or tissue culture. Thus the transgenic plants may be used to generate one or more generations of descendants. For example, the dwarf transgenic plants may be introduced into standard plant breeding programmes. Some of these descendants will display modified growth characteristics and may be selected on the basis of this phenotype.

The DNA construct used for transformation includes a DNA sequence encoding the functional phytoene synthase enzyme and a transcriptional initiation region (or promoter) operative in plants, so that the construct can generate mRNA in plant cells. Both constitutive promoters (such as the 35S cauliflower mosaic virus promoter) and inducible or developmentally regulated promoters (such as the CAB promoter driving expression in green tissues) may be used as circumstances require. Use of a constitutive promoter will tend to affect functions in all parts of the plant, while use of a tissue specific promoter allows more selective control of gene expression and affected functions. Use of the phytoene synthase gene promoter itself (International application WO92/16635), at least in tomatoes, has the advantage that the production of increased amounts of the enzyme occurs at a time when carotenoids are normally synthesised.

Plant growth characteristics may be modified to a greater or lesser extent by controlling the degree of mRNA production in the plant cells. This may be done by suitable choice of promoter sequences, or by selecting the number of copies or the site of integration of the phytoene synthase DNA sequences that are introduced into the plant genome. For example, the DNA construct may include more than one DNA sequence encoding phytoene synthase or more than one recombinant construct may be transformed into each plant cell.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used.

It is convenient to use a cDNA clone as the DNA sequence. For example, the base sequence of TOM5 cDNA (inserted into plasmid pTOM5) is disclosed in Ray et al, 1987, Nucleic Acids Research, 15:10587 and in international application WO91/09128. This clone was deposited as a plasmid in E.coli on 1 September 1989 under the terms of the Budapest Treaty at the National Collections of Industrial and Marine Bacteria (23 St. Machar Drive, Aberdeen, AB2 1RY, Scotland) and was given the reference NCIB 40191. A cDNA clone similar to pTOM5 may alternatively be obtained from the mRNA of ripening tomatoes by the method described by Slater et al (1985, Plant Molecular Biology, 5:137-147).

An alternative source of DNA for the base sequence for transcription is a suitable gene encoding the phytoene synthase. This gene may differ from the cDNA in that introns may be present. The introns are not transcribed into mRNA (or, if so transcribed, are subsequently cut out). When using such a gene as the source of a partial base sequence for transcription it is possible to use either intron or exon regions. For example, the TOM5 gene was deposited as a plasmid (gTOM5) in phage 1 EMBL3 on 11 March 1991 under the terms of the Budapest Treaty at the National Collections of Industrial and Marine Bacteria (23 St. Machar Drive, Aberdeen, AB2 1RY, Scotland) and was given the reference NCIMB 40382. International Patent Publication number WO92/16635 discloses the structure of the TOM5 gene and nucleotide sequences of the 3.5 kb EcoRI - SalI fragment and the 3' region of the gTOM5 phytoene synthase gene.

A further way of obtaining a suitable DNA base sequence for transcription is to synthesise it ab initio from the appropriate bases, for example using the pTOM5 or gTOM5 sequences as a guide.

The preferred DNA sequence for use in the present invention is DNA derived from the cDNA clone pTOM5 or the genomic clone gTOM5 or a synthetic polynucleotide based on the pTOM5/gTOM5 sequence. However, other sources of phytoene synthase genes may also be used as suitable genes may be isolated from other plant species or from bacteria, yeast, lower and higher eukaryotes.

To obtain vectors suitable for expression of the functional phytoene synthase in plant cells, the complete cDNA sequence or the complete gene sequence as found in the chromosome of the plant may be used. Recombinant DNA constructs may be made using standard techniques. For example, the DNA sequence for transcription (encoding phytoene synthase) may be obtained by treating a vector containing said sequence (such as pTOM5) with restriction enzymes to cut out the appropriate segment. The DNA sequence for transcription may also be generated by annealing and ligating synthetic oligonucleotides or by using synthetic oligonucleotides in a polymerase chain reaction (PCR) to give suitable restriction sites at each end. The DNA sequence is then cloned into a second vector containing the desired upstream promoter and downstream terminator sequences. Suitable promoters include the 35S cauliflower mosaic virus promoter and the tomato polygalacturonase gene promoter sequence (Bird et al, 1988, Plant

Molecular Biology, 11:651-662). Suitable terminator sequences include that of the Agrobacterium tumefaciens nopaline synthase gene (the nos 3' end).

The invention will now be described by way of example only with reference to the accompanying drawings, in which:

Figure 1 shows the pathway of carotenoid biosynthesis.

Figure 2 is a diagram showing the construction of a phytoene synthase over-expression vector, pBDH5ST.

Figure 3 is a graph showing the height of transgenic seedlings.

Figure 4 is a diagram showing part of the isoprenoid pathway.

Figure 5 is a graph showing the mean internode lengths of homozygous, hemizygous and azygous plants.

Figure 6 is a graph showing the levels of gibberellins in a control plant and a transformant.

Figure 7 is a graph showing the level of GA1 in a control plant and a transformant.

EXAMPLE 1

Characterisation of the TOM5 phytoene synthase gene family.

The cDNA clone pTOM5 encodes (almost completely) a plant gene which expresses an enzyme involved in the ripening of tomatoes (Ray et al, 1987, Nucleic Acids Research, 15:10587). pTOM5 was derived from a cDNA library isolated from ripe tomato RNA (Slater et al, 1985, Plant Molecular

Biology, 5:137-147). Three other clones (pTOM45, pTOM91, pTOM104) from the same library cross-hybridise to pTOM5 and probably contain related sequences. pTOM5 has been characterised by hybrid select translation to encode a protein of approximately 48kD. DNA sequence analysis has demonstrated that the clone is 1600 bases long with an open reading frame encoding a polypeptide of 46.7kD. The mRNA for which pTOM5 codes is expressed in ripening tomato fruit. No expression could be detected in green fruit. pTOM5 is expressed most strongly at the full orange stage of ripening. The level of mRNA then declines in line with the general decline in biosynthetic capacity of the ripening fruit. Expression of pTOM5 mRNA could also be induced by exposing mature green fruit to exogenous ethylene. The expression of pTOM5 is reduced in the Ripening inhibitor (rin) and Neverripe (Nr) tomato fruit ripening mutants which mature very slowly, and never achieve the full red colour associated with ordinary tomato fruit.

Biochemical experiments have established that the pTOM5 gene product is the phytoene synthase enzyme (international application WO92/16635). Tomatoes in which the expression of the pTOM5 gene was inhibited using antisense RNA no longer accumulate carotenoids, in particular phytoene. Biochemical feeding experiments with these tomatoes demonstrated that phytoene synthase was inhibited in the fruit.

International application WO92/16635 describes the isolation of pTOM5-related genes. The genomic locations in the tomato of sequences homologous to

pTOM5 were identified using RFLP mapping: two loci, on chromosome 2 and chromosome 3 respectively, carry sequences homologous to pTOM5. It was also shown by Southern blotting that the gene may be present as a small multigene family. Genomic clones representing two individual genes were isolated and characterised by DNA sequence analysis. The clone gTOM5 represents part of a gene with exon sequence identical to pTOM5. Clone F contains a sequence similar, but not identical to pTOM5. The genomic clones cover most of the coding region and the complete transcriptional initiation region of the phytoene synthase gene.

EXAMPLE 2

Construction of phytoene synthase over-expression vectors with the CaMV 35S promoter

(a) pJR15CS

Bases 1 to 1598 (the complete cDNA) of pTOM5 were made into a PCR-generated fragment digested with KpnI and BamHI. The cut ends were made flush with T4 polymerase and then cloned into the HincII site of pJR1. pJR1 (Smith et al, 1988, Nature, 334:724-726) is a Bin19 (Bevan, 1984, Nucleic Acids Research, 12:8711-8721) based vector which permits the expression of the pTOM5 RNA under the control of the CaMV 35S promoter and has a nopaline synthase (nos) 3' end termination sequence. After synthesis, the vectors with the sense orientation of pTOM5 sequence were identified by DNA sequence analysis. This vector was called pJR15CS.

(b) pBDH5ST

Figure 2 is a diagram showing the construction of the phytoene synthase over-expression vector, pBDH5ST.

The SspI fragment of pTOM5 was cloned into SmaI-cut pT7T3α18 to form the vector pT7T3TOM5. pT7T3TOM5 was sequentially treated with EcoRI, Klenow then BamHI to remove the TOM5 insert. This was ligated into pDH51 (previously treated with SalI, Klenow and BamHI) to form the vector pDH5ST (CaMV 35S promoter and terminator). The EcoRI fragment from pDH5ST was cloned into the EcoRI site of pBIN19 to form pBDH5ST.

The pBDH5ST-type construct is more effective in producing the dwarf phenotype.

EXAMPLE 3

Generation of transformants and selection of dwarf plants

pBDH5ST vectors were transferred to Agrobacterium tumefaciens LBA4404 and used to transform tomato plants. Transformation of tomato stem segments followed standard protocols (Bird et al, 1988, Plant Molecular Biology, 11:651-662). Transformed plants were identified by their ability to grow on media containing the antibiotic kanamycin. Plants were regenerated and grown to maturity.

Transformed plants showing the altered growth phenotype (dwarf plants with reduced internode lengths) were selected.

The introduced TOM5-derived construct expresses phytoene synthase. At the same time, it may cause some suppression of the endogenous phytoene synthase (TOM5) gene. A larger proportion of the transformed plants may be dwarf if co-suppression were prevented so that reproducibly high levels of enzyme over-expression could be obtained. Co-suppression in tomato may not occur if a phytoene synthase gene from a different organism were used in the construct.

EXAMPLE 4

Analysis of dwarf plants

The selected dwarf transgenic plants show an altered growth habit (reduced height, reduced internode lengths). In general, the selected plants also showed the following characteristics:

the seeds germinated more slowly (germination of the seeds can be improved by pre-treatment with sodium hypochlorite; alternatively germination may be improved by pre-treatment with gibberellin);

the roots of the germinating seedlings had a pale pink/red colour;

the seedlings were shorter.

Table 1 compares the phenotype of one of the homozygous dwarf lines (designated BDH5STB) with an azygous plant derived from the progeny of the same primary transformant.

TABLE 1

NUMBER OF PLANTS		
Distance from cotyledon to first true leaf (mm)	Homozygous TOM5 over-expresser (line BDH5STB)	Azygous seedlings not inheriting the TOM5 transgene
0-9	11	0
10-19	9	2
20-29	3	3
30-39	0	6
40-49	0	6

Table 2 shows measurements from mature 5 month old plants of the dwarf line BDH5STB and of a second dwarf line BDH5ST:Z171. (Internodes are numbered down from the apex and distances are in millimetres). This data demonstrates the reduced internode lengths. The dwarf phenotype is quite obvious, with a mean reduction of slightly greater than ten fold for internodes 3-6.

TABLE 2

	DISTANCE (millimetres)			
Internode number	First control	Second control	BDH5ST line B	BDH5ST line Z171
1	3	2	2	3
2	5	5	3	2
3	31	29	2	3
4	34	41	2	11
5	42	44	6	6
6	43	73	3	3

Other dwarf lines showed more severe dwarfing and large numbers of seedlings from three different lines are being germinated for additional synchronised measurements.

EXAMPLE 5

Further analysis of dwarf plants

Transgenic tomato plants have been produced in which transcription of a fruit phytoene synthase cDNA, TOM5 (containing all of the translated sequence), is driven by a constitutive CaMV 35S promoter. Some of these plants were capable of producing a functional phytoene synthase protein as judged by their ability to complement a naturally deficient mutant and by the increased carotenoid content of immature fruit and senescent leaves of

both mutant and wild type plants carrying the transgene. Over-expression of phytoene synthase in some plants altered hormone synthesis resulting in dwarfing. Internode length was much reduced (resulting in dwarf plants): a six week old seedling that over-expresses the TOM5 transgene was shorter than a wild type plant of similar age. Figure 3 is a graph showing the height of seedlings from seven different transgenic lines (labelled Z171 D3-E9). The seedlings from line D9 showed approximately the same height and growth pattern as those of wild type (AC++). The seedlings from line D4 were taller than the control. However, the seedlings from lines D3, D5, E3, E4 and E9 showed varying degrees of retarded growth relative to those of wild type (AC++). The dwarf phenotype persisted through at least four months growth and an apparent loss of apical dominance occurred in some cases, leading to a bushy phenotype. Leaf size was also reduced. Similar phenotypes (though varying in severity) have been found in the progeny of several independently transformed over-expressing lines, both of transgenic wild-type and yellowflesh mutant plants. This dwarf phenotype is similar to that seen in plants that have been treated with inhibitors of gibberellic acid synthesis.

The isoprenoid pathway produces the precursors of three major classes of plant growth regulators - cytokinins, gibberellins (GA) and abscisic acid (ABA). Figure 4 illustrates part of this pathway. Many plant volatiles are due to terpenes and the phytol side chain of chlorophyll is also formed from isoprenoid units. Altering the metabolism of any intermediate in this pathway may have profound

effects on the levels of many important plant products. A detailed analysis of the dwarf plants expressing the TOM5 construct is currently underway. However, the synthesis of phytoene from two molecules of geranylgeranyl diphosphate could redirect pools of this precursor away from the synthesis of gibberellins. If increased levels of phytoene result in an increased flux through the down stream pathway, higher levels of abscisic acid might also result. Reduced gibberellins and/or increased abscisic acid levels may be the primary cause of the observed dwarf phenotypes. Preliminary measurements indicate that gibberellins are reduced and abscisic is increased in tissues from these dwarf plants. The severity of the stunted phenotype correlates well with the level of expression of the transgene as determined by mRNA analysis. The degree of internode shortening in mature plants correlates well with the level of expression (as determined by northern analysis) of the TOM5 transgene in homozygous, hemizygous and azygous progeny of a primary transformant containing a single insert. Figure 5 is a graph showing the mean internode lengths of homozygous plants (highest level of TOM5 mRNA), hemizygous plants (intermediate level of TOM5 mRNA) and azygous plants (no TOM5 transgene mRNA).

Figure 6 is a graph showing the levels of different gibberellins (GAs) in a control plant and a line expressing a phytoene synthase transgene (BDH5ST). Several gibberellins were reduced in TOM5 over-expressers, including a 30-fold reduction in the active GA (shown by the graph in Figure 7).

Other (non-dwarf) transformants in which the presence of the transgene (TOM5 constructs) resulted in co-suppression of the fruit, flower and leaf phytoene synthase genes, had a growth habit with some characteristics of GA treated plants. They were taller than control plants and had thinner, more fragile stems with longer internode distances. This phenotype could be explained if the inhibition of an endogenous phytoene synthase has resulted in more available geranylgeranyl diphosphate which in turn has been converted to various gibberellins. The germination rate of seeds from the sense-suppressed plants was increased and on occasion germination actually occurred within the fruit. Increased GA or decreased ABA may play a role in such a phenotype.

CLAIMS

1. A method to produce plants having modified growth characteristics which comprises transformation of target plants with a DNA construct including a DNA sequence expressing phytoene synthase, growth of transformed plants, and selection of plants having modified growth characteristics.

2. A method as claimed in claim 1 in which the plants having modified growth characteristics are dwarf plants.

3. A method as claimed in either claim 1 or claim 2 in which the DNA sequence is a TOM5 sequence.

4. A method as claimed in claim 3 in which the DNA sequence is a TOM5 cDNA sequence.

5. A plant having modified growth characteristics which comprises a plant cell transformed with a DNA construct including a DNA sequence expressing phytoene synthase.

6. A plant as claimed in claim 5 which is a dwarf plant.

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FIG. 1

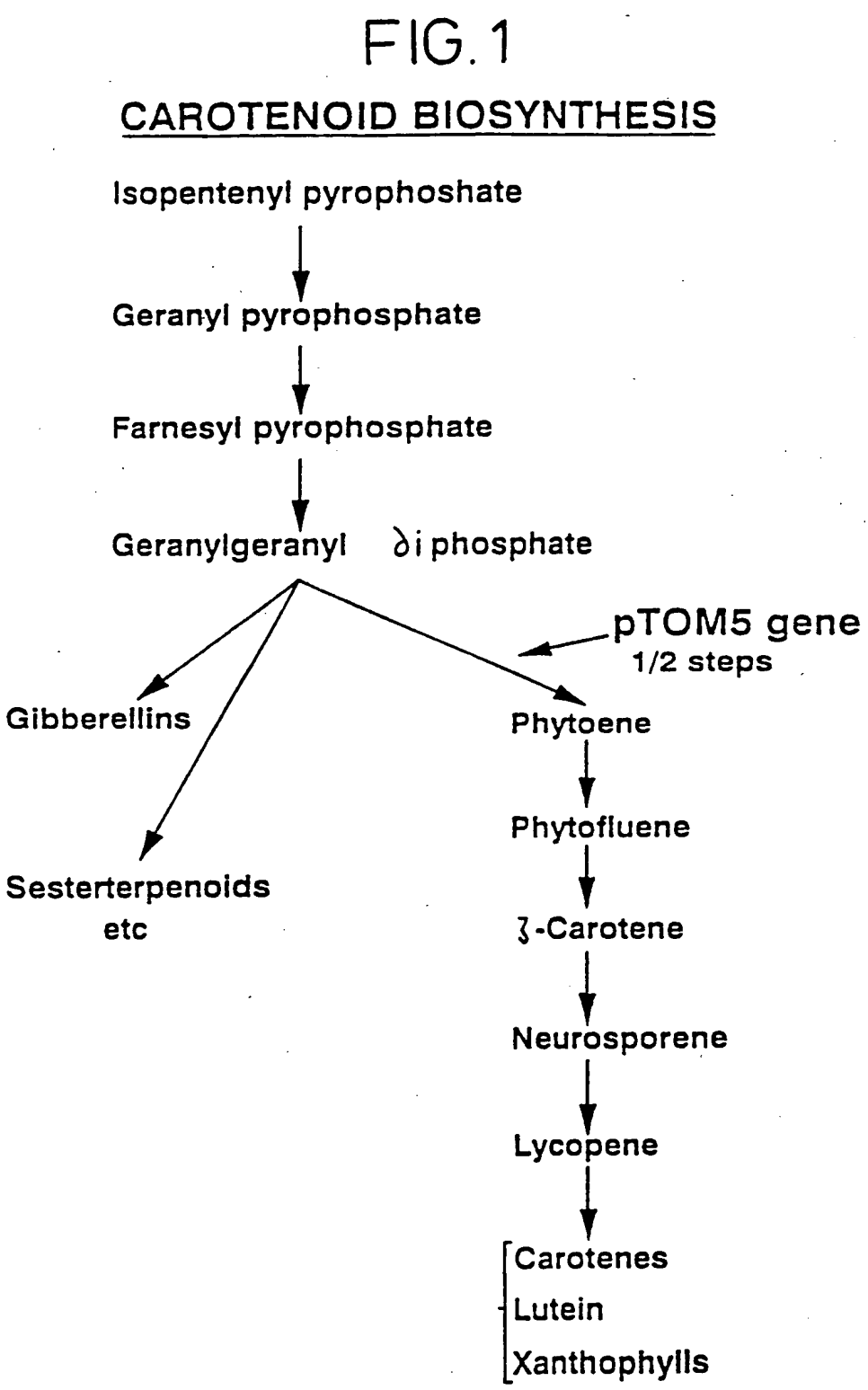
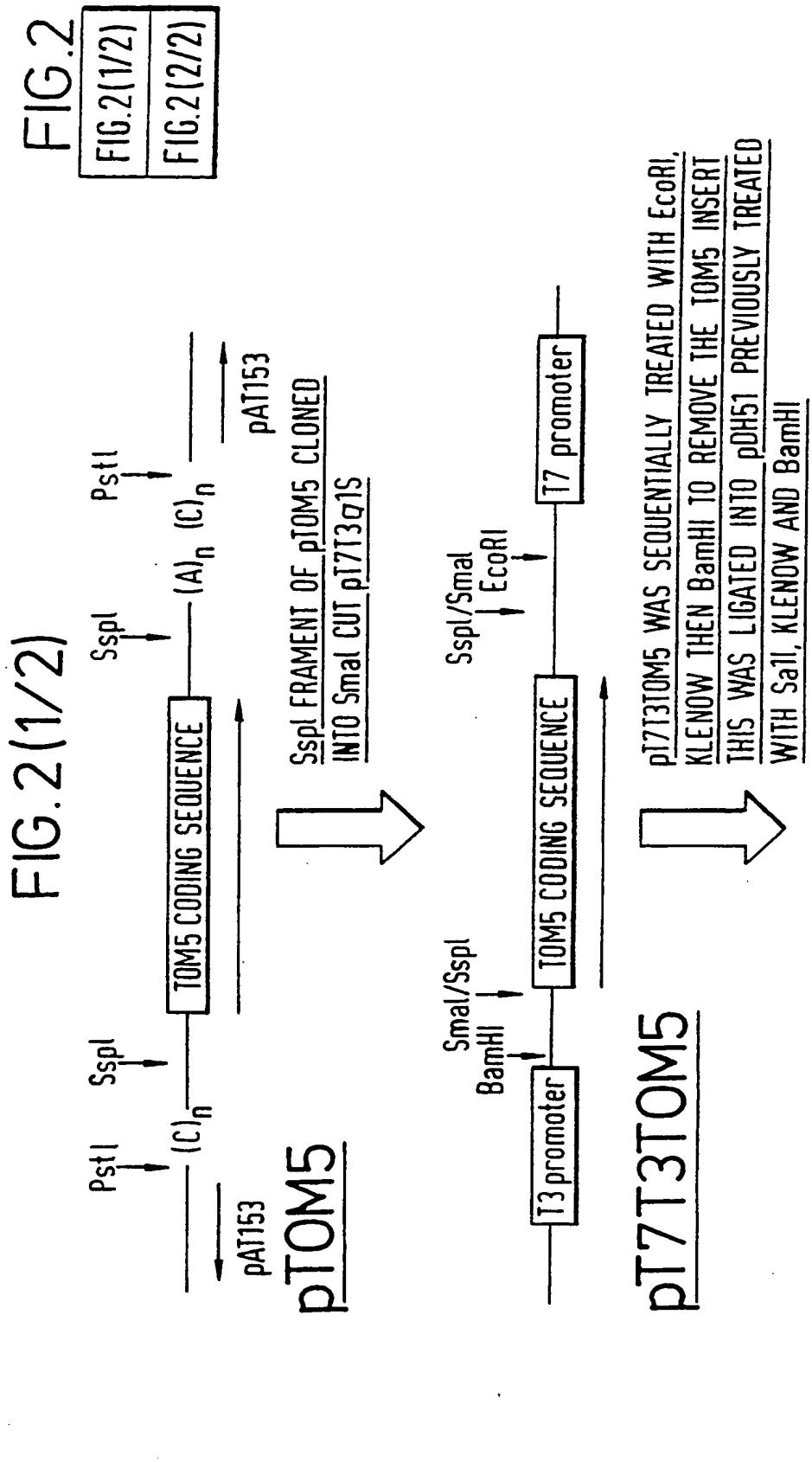

CAROTENOID BIOSYNTHESIS
Isopentenyl pyrophoshate
Geranyl pyrophosphate
Farnesyl pyrophosphate
Geranylgeranyl ∂i phosphate
pTOM5 gene
1/2 steps
Gibberellins
Sesterterpenoids
etc
Phytoene
Phytofluene
ζ-Carotene
Neurosporene
Lycopene
Carotenes
Lutein
Xanthophylls

FIG.2

FIG.2(1/2)

FIG.2(2/2)



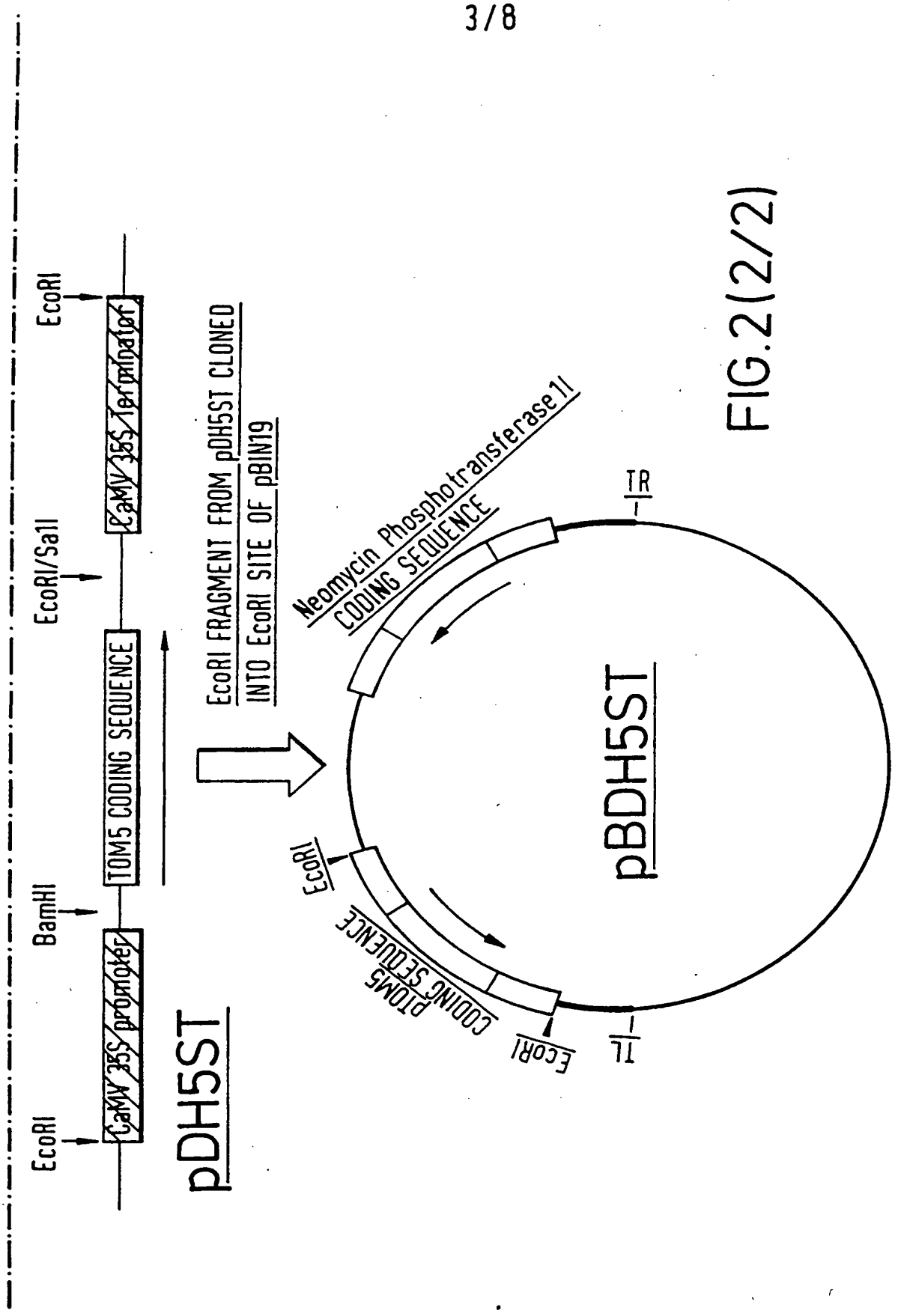

pDH5ST
EcoRI
CaMV 35S promoter
BamHI
TOM5 CODING SEQUENCE
EcoRI/SalI
CaMV 35S terminator
EcoRI
EcoRI FRAGMENT FROM pDH5ST CLONED
INTO EcoRI SITE OF pBIN19
pBDH5ST
Neomycin Phosphotransferase II
CODING SEQUENCE
TR
EcoRI
TOM5
CODING SEQUENCE
EcoRI
TL

FIG.2(2/2)

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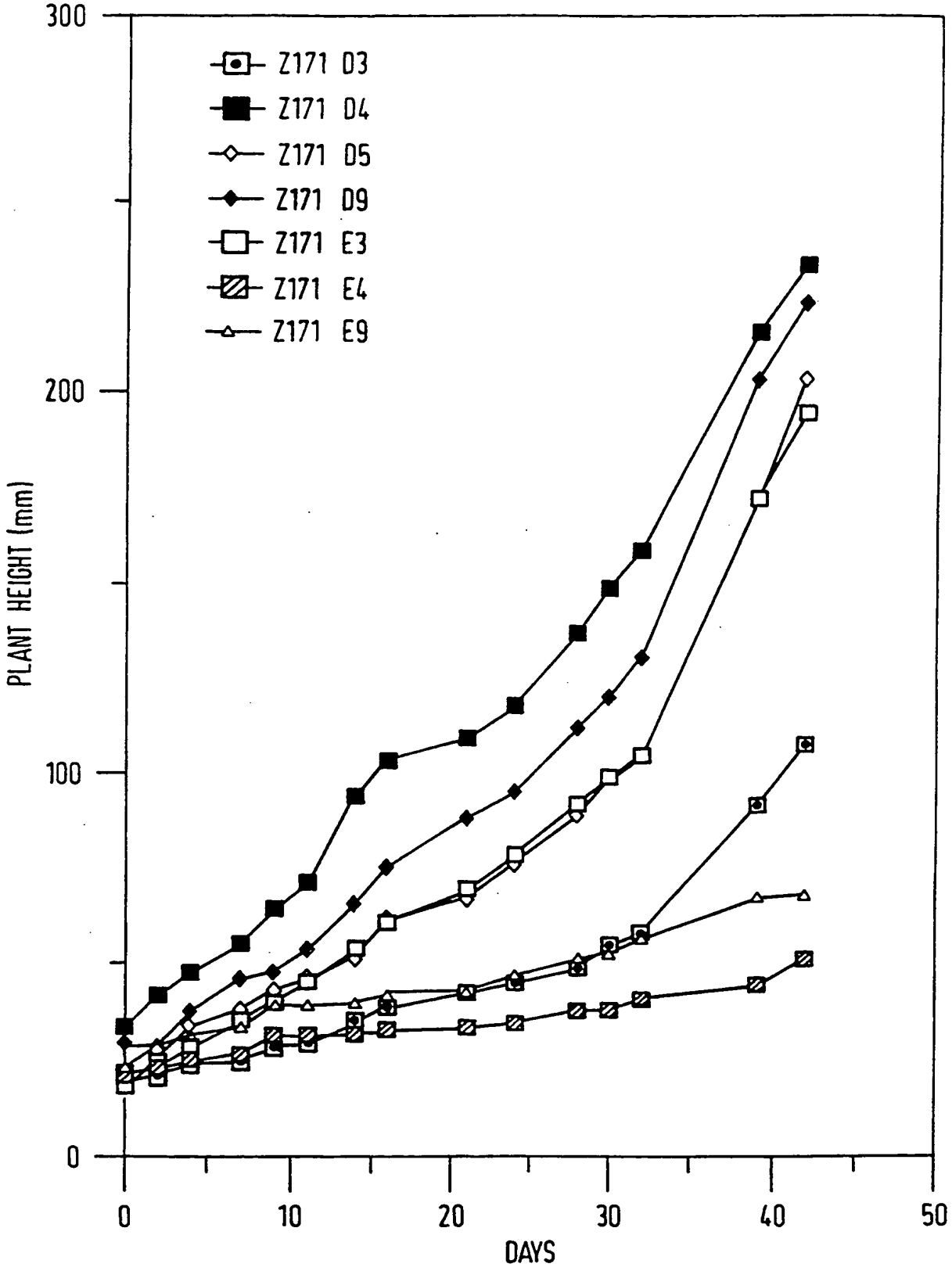
FIG.3


Z171 D3
Z171 D4
Z171 D5
Z171 D9
Z171 E3
Z171 E4
Z171 E9
PLANT HEIGHT (mm)
300
200
100
0
0
10
20
30
40
50
DAYS

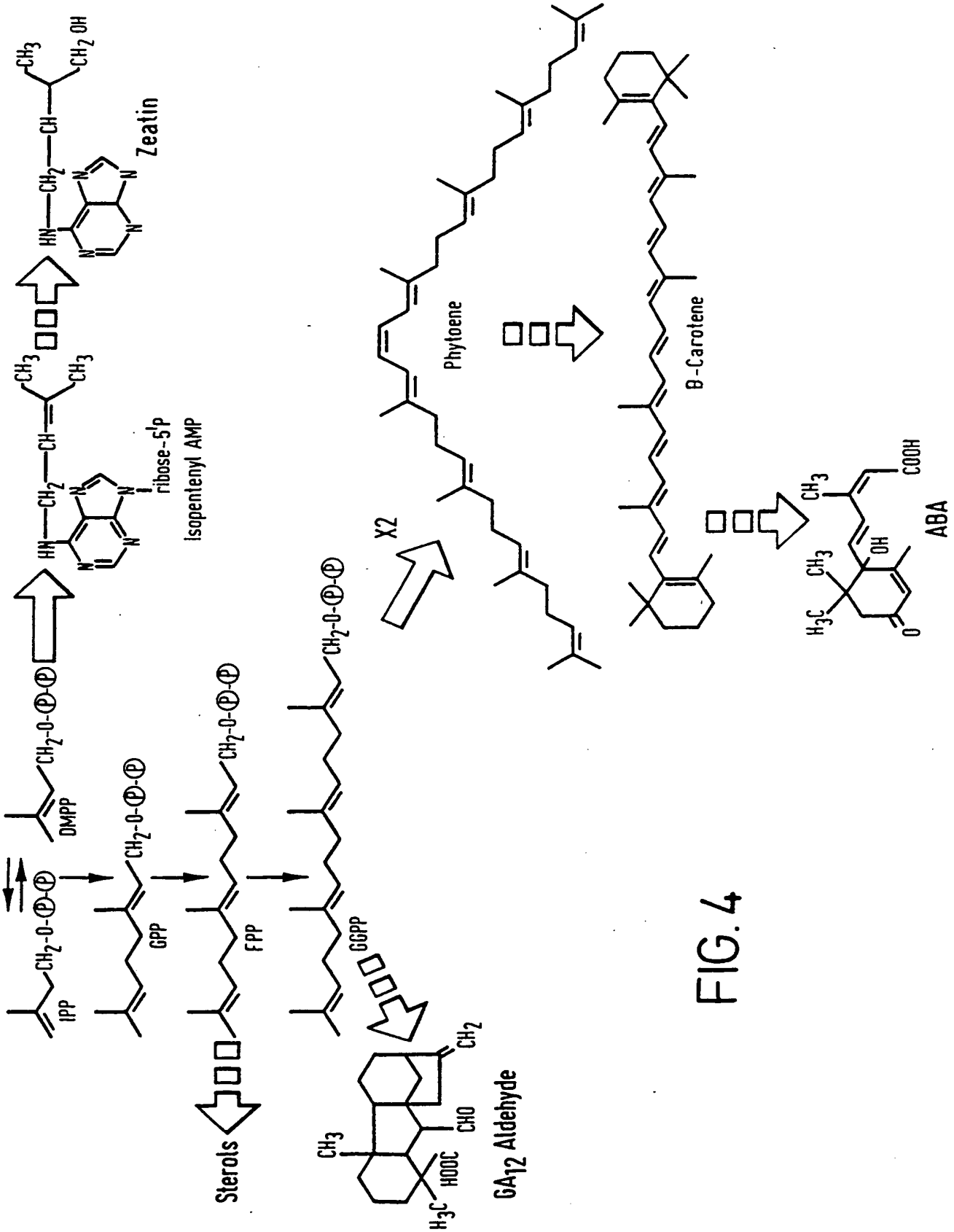

IPP
DMPP
GPP
FPP
GGPP
Sterols
GA12 Aldehyde
Isopentenyl AMP
ribose-5'p
Zeatin
X2
Phytoene
B-Carotene
ABA

FIG. 4

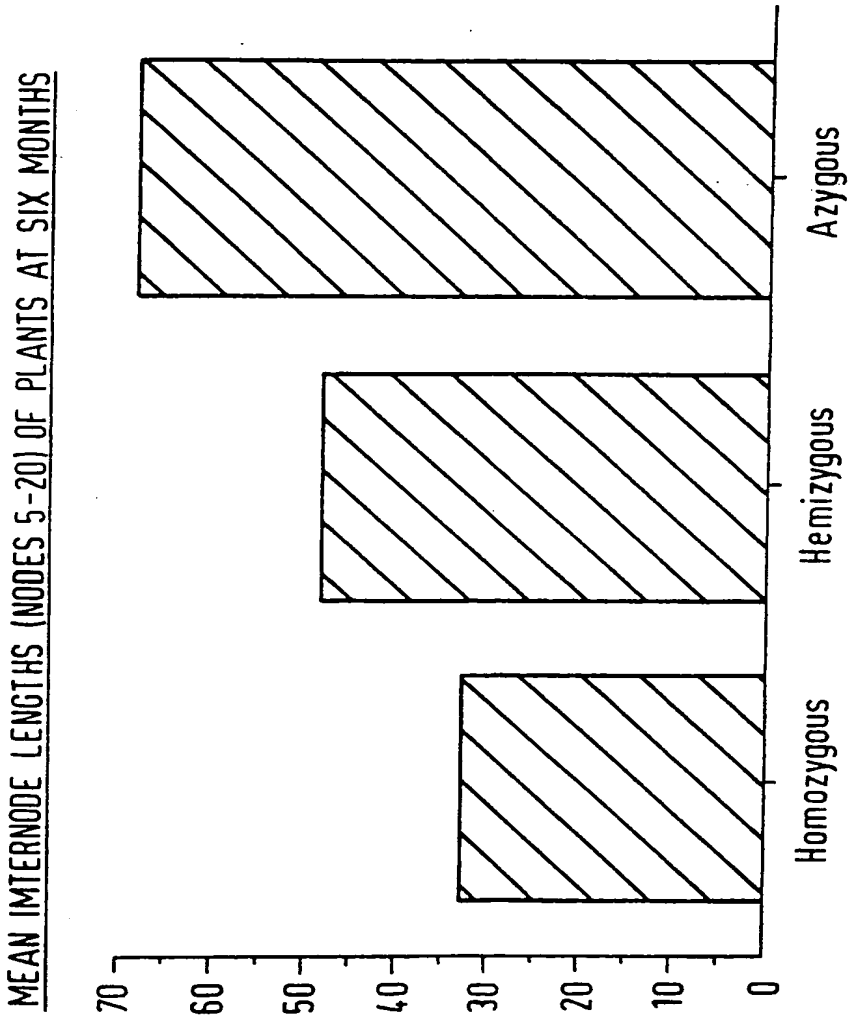

MEAN IMTERNODE LENGTHS (NODES 5-20) OF PLANTS AT SIX MONTHS
70
60
50
40
30
20
10
0
Homozygous
Hemizygous
Azygous

FIG.5

FIG.6

LEVELS OF DIFFERENT GAs

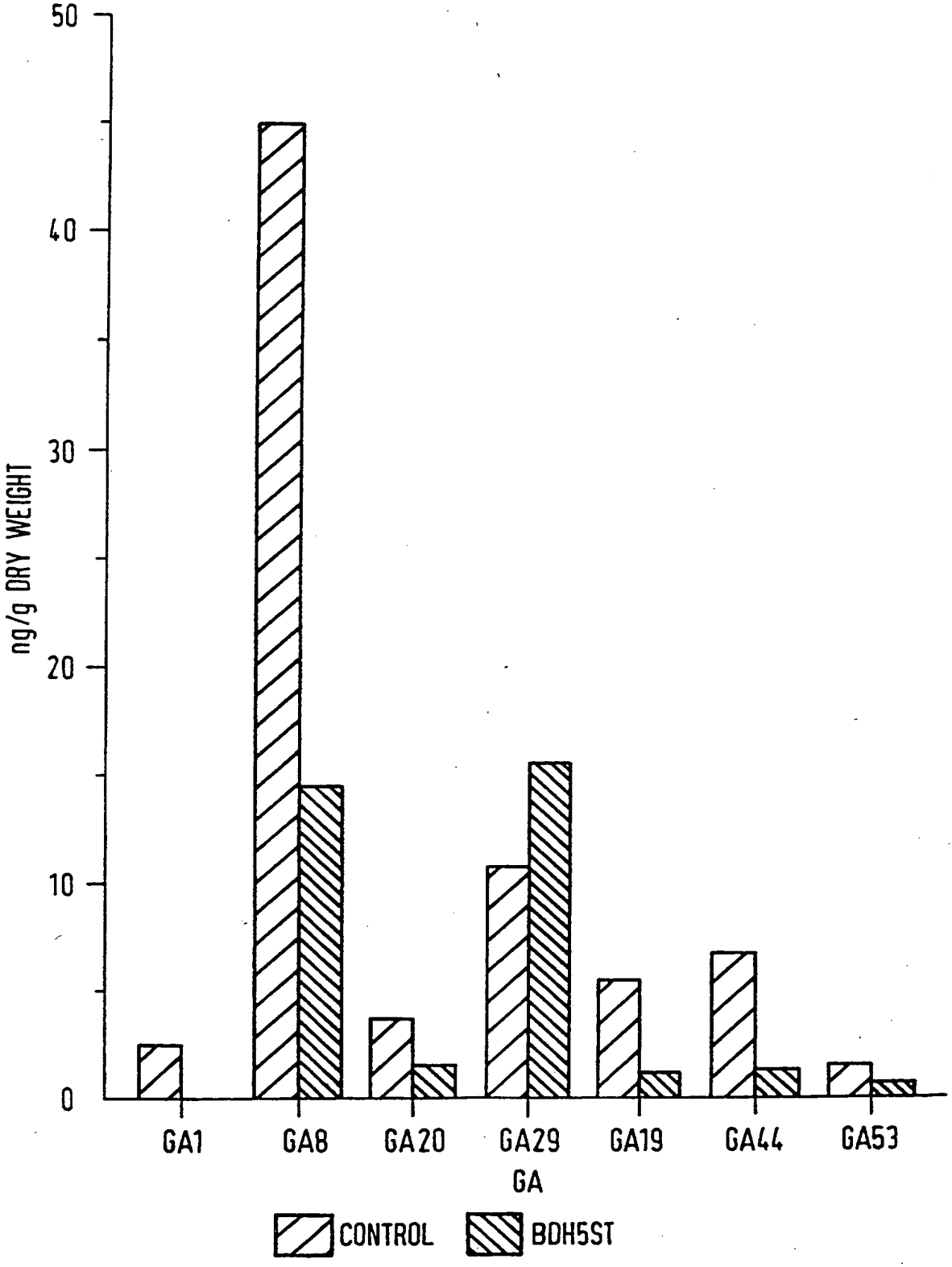
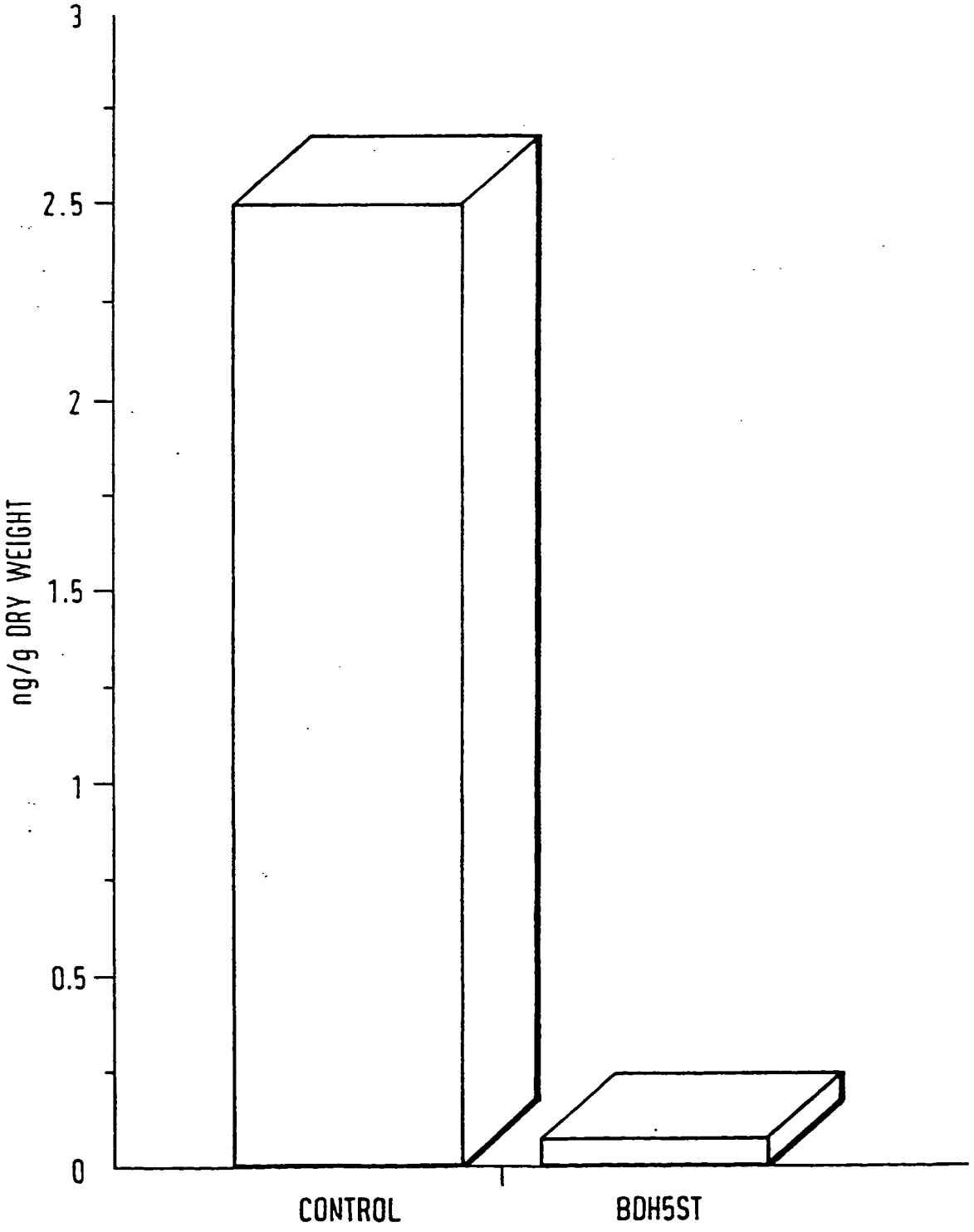


FIG.7

GA1 LEVELS IN A LINE EXPRESSING A PHYTOENE SYNTHASE TRANSGENE



INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 94/01465

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 A01H5/00 C07K14/415

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 16635 (IMPERIAL CHEMICAL INDUSTRIES PLC) 1 October 1992 cited in the application see whole document. ---	1
A	WO,A,91 09128 (IMPERIAL CHEMICAL INDUSTRIES PLC) 27 June 1991 cited in the application see whole document. ---	1
A	WO,A,91 13078 (AMOCO CORPORATION) 5 September 1991 see Examples 3, 7 and 18, Figure 1 and claims. -----	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
11 November 1994	06. 12. 94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Yeats, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/GB 94/01465

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9216635	01-10-92	AU-A-	1369092	21-10-92
		BR-A-	9205770	07-06-94
		EP-A-	0619844	19-10-94
		JP-T-	6505871	07-07-94
WO-A-9109128	27-06-91	AU-B-	645534	20-01-94
		AU-A-	6893891	18-07-91
		EP-A-	0505405	30-09-92
		JP-T-	5502160	22-04-93
		US-A-	5304478	19-04-94
WO-A-9113078	05-09-91	EP-A-	0471056	19-02-92
		JP-T-	5504686	22-07-93